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Dietetic Value of Salsify

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THE DIETETIC VALUE OF SALEP

by

Louise McDanell

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Submitted in partial fulfilment of the requirements

for the degree of

Master of Arts

in the Faculty of Philosophy

Columbia University

THE AESTHETIC VALUE OF SAINT

by

Lorine McDermott

Submitted in partial fulfillment of the requirements

for the degree of

Master of Arts

in the Faculty of Philosophy

Columbia University

The Dietetic Value of Salep

Salep belongs to a large class of substances yielding vegetable gums, to which the name hemicellulose is given. Other sources of these gums are Iceland Moss, Irish moss, slippery elm, and the Hawaiian and Japanese edible seaweeds. Agar agar and the so-called "vegetable gelatin" are well known forms.

These carbohydrates are distinguished from cellulose by being capable of hydrolysis on boiling with dilute mineral acids, and from other polysaccharide carbohydrates by not being readily digested by diastase.

There seems to be a special value attached to these mucilages in the popular mind. The prevailing impression is shown in some of the following remarkable statements. The United States Dispensatory, 1908, not only says that the mucilaginous extract of slippery elm bark (Ulmus fulva, Michaux) is nutritious, but adds, "We are told that it has proved sufficient for the support of life in the absence of other food!" Of salep, Smith (27) says, in his dictionary of economic plants: "It contains a chemical substance called bassorin which is said to contain more nutritious matter than any other vegetable product, one ounce per diem being sufficient to sustain a man"! The United States Dispensatory also assures us that salep is "highly nutritious"! Johnson (17) particularly recommends Iceland moss (Cetraria islandica) as a diet for consumptives, as "it seems to be both extremely nutritious and very easy of digestion, though of course only capable of use as a substitute for starchy matters"! In regard to Irish moss (Chrondus crispus), he is a little more uncertain, saying, "It is much used for invalids, especially in cases of consumption, but with doubtful advantage when substituted for more nutrititious food"

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Schneider (25) says of Iceland moss: "Inhabitants of Iceland, Norway, and Sweden mixed this lichen with various cereals and mashed potatoes, from which an uncommonly healthful bread was prepared". After a few metabolism experiments, Oshima (20) remarks, concerning the algae of Japan: "Their actual value doubtless depends in considerable measure upon the mineral salts they contain".

Historical

It was in view of the scarcity of scientific investigation as to the behavior in the animal body of this large class of substances and of their wide distribution and use that Miss Mary Davies Swartz undertook their investigation at the laboratory of physiological chemistry of the Sheffield Scientific School of Yale University in 1908. An account of her work is published in the Transactions of the Connecticut Academy of Arts and Sciences,vol.16, pp. 247-382, 1911, under the title, "Nutrition Investigations on the Carbohydrates of Lichens, Algae, and Related Substances". This article contains a detailed review of the literature and the work done up to that time, and, as little has been done since, most of such material herein given is taken from that paper.

Commercial salep is obtained from the tubers of certain species of Orchis and Eulophia, native to Turkey, the Caucasus, Asia Minor, and the greater part of Central and Southern Europe. The small ovoid, oblong, or palmate tubers are decorcitated, washed, heated until horny and semi-transparent, and finally dried. An abundant mucilaginous extract is obtained by macerating the bulbs in water. Frequently the bulbs are ground to powder, and the powder used like sago or tapioca. Royal salep said to be used as food in Afghanistan is prepared from Allium Macleanii.

A former instructor in the American College for Girls in Constantinople reports that salep is a very common article of diet in Turkey. It is sold in the markets in powdered form, and is made into a sort of sweetened gruel with milk. Not only is it used as a warm drink in the household, much as we use cocoa or chocolate, but it is also sold in the streets by venders, who either stand in booths along the way, or go about carrying huge brass urns strapped to their shoulders, clinking their cups and calling "Taze-Sahlep!" (Fresh Salep). It is especially popular in districts of the city where people work late at night. It is no uncommon thing to see the workman standing with his salep cup in hand, waiting for the firing of the sunset cannon.

The tubers are rich in mannan, easily hydrolyzed by dilute acids, yielding the hexose, mannose, but not hydrolyzed by diastatic enzymes. The mucilaginous material obtained on extraction with water was first studied by C.Schmidt (24) in 1844, and called by him "salep-bassorin" On hydrolysis with dilute sulphuric acid, he obtained, besides some gummy substance and cellulose, a fermentable sugar which he thought to be dextrose. Mulder (19) considered the salep mucilage a mixture of starch ad gum or pectin acids, while Franck (14) thought it a modification of cellulose, and Girand (15) a transformation of a starchy substance into a variety of dextrin swelling in water. Pohl (21) by precipitation with mineral salts, distinguished an "α-Schleim" and a "β-Schleim" According to Thamm (28), who has made the most recent investigations, "α-Schleim" does not occur in German salep. Tollens and Gans (29) showed that on hydrolysis, besides dextrose, mannose, or as they called it, "isomanitose" was formed, but this was shown by Fischer and Hirschberger (13) to be identi-

cal with d-mannose. Tham (28) and Hilger (16) have shown conclusively that the starch-free water extract contains an anhydride of mannose only.

Bacteria, enzymes, and molds capable of converting mannan into mannose are known to exist in the vegetable kingdom, notably in the date embryo, carob seeds, and the seeds of certain legumes, at least at the time of germination. There are a few instances on record of mannanases in lower animals.

Previous to the experiments at Yale, referred to above, there were few records of feeding experiments with mannan. An experiment by Hauber on a dog, was reported by Voit (30) in 1874. Three hundred ninety grams of dry salep powder was fed to a medium sized dog in eight days. It was a crude experiment, but calculations showed that at least 50 per cent of the salep was absorbed. In 1879, Weiske (31) compared carob beans (Ceratonia siliqua) with other food for sheep, and as a result of his experiments, pronounced "Johannis-brod" (carob beans) a digestible and acceptable sheep-food. In 1890, Shuster and Liebscher (26) having found that the sawdust of the ivory nut (Phytelepas macrocarpa) had a favorable effect on cattle, tried feeding it to sheep, and reported it satisfactory From these experiments, mannan would seem to be well utilized by herbivora. The only experimental data regarding the nutritive value of mannan to man are cited by Oshima (20) from work by Kano and Iishima (18), who found the coefficient of digestibility of konjaku 82 per cent, (prepared from Conophallus konjaku).

It remained to be determined whether mannan can be hydrolyzed within the organism, and if so, whether the mannose produced can be retained and form glycogen. From the literature on the subject, it

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appears that mannose is well utilized by rabbits, dogs, and men. That mannose can act as a glycogen former in rabbits has been demonstrated by Cremer (12) and also by Rosenfeld (22). There is good reason for assuming, therefore, that if mannan can be converted into mannose in the process of digestion, it may be considered as a true nutrient for the organism, the mannose being to a high degree capable of absorption and conversion into glycogen.

The mannan from salep was included among the hemicelluloses investigated by Swartz (10), and the results obtained are here summarized.

It was found unattacked by the enzymes of saliva, malt diastase, pancreatic extract, and pancreatic juice, and intestinal juice. "Taka" diastase affected it in three days. Gastric extract was effective, but as it was hydrolyzed similarly by 0.2 per cent hydrochloric acid, the enzyme cannot be held responsible for the action.

In experiments with bacteria, incubation for three days gave negative results, and apparently all the material was recovered unaltered, except in certain instances where the salep underwent an insoluble modification. In trials where the cultures were allowed to grow one to three weeks, no difference could be detected. Marked evidences of change were observed in some of the four-week cultures. Sawamura (23) thought that he observed a slight hydrolysis of mannan by B. Prodigiosus, an observation not verified in these experiments. No reducing substance was detected in the three-day cultures or the four-week cultures in which this organism was present. The opaque jelly, insoluble in water, formed from salep by the action of B. Coli communis, B. Prodigiosus, and mixed cultures containing these organisms, resembled an intermediary product of the acid hydrolysis of salep-mannan described by Thamm (28).

It seems reasonable to inquire whether this insoluble material produced by bacterial action may not be regarded as an early stage in the hydrolysis of the carbohydrate, especially in view of the fact that in all other four-week trials a very definite reduction of Fehling's solution was noted, corresponding in strength with the loss of unaltered carbohydrate, as shown by the following summary:

Salep

Cultures Used	Reduction of Fehling's Solution	Rotation of Unaltered carbohydrate after Hydrolysis	
		Salep	Control
Mixture of Pure Aerobes	(Insoluble jelly)	Not determined	
Mixture of Faecal and Soil Bacteria (aerobic)	+	+0.17°	+0.20°
Mixture of the Bacilli of Malignant Oedema and Symptomatic Anthrax	+	+0.13°	+0.20°
Mixture of Faecal and Soil Bacteria (anaerobic)	+	+0.03	+0.20°

From Swartz, page 331.

These experiments give some grounds for expecting the hydrolysis of salep in the alimentary tract through the action of bacteria.

Subcutaneous and intraperitoneal injections were made with dogs. After these, the hemicellulose was excreted through the kidneys, and recovered unaltered in the urine. The carbohydrate of salep was completely eliminated in one to three days.

Feeding experiments showed that those hemicelluloses most readily attacked by bacteria disappear most completely from the alimentary tract. Seven experiments were made. In four, the commercial salep powder (containing 19 per cent mannan and 26 per cent starch) was administered; in the others, pure salep-mannan prepared from orchis tubers. The results are tabulated by Swartz on pages 354 to 356, from which Table 1 is compiled. These results show that in every case, except that in which a dog received in one day ten grams of pure mannan, the greater portion of the salep fed was digested, the coefficient of digestibility of salep powder for dogs averaging 85 per cent, and for man 97 per cent; while that of pure mannan for man was 100 per cent, in spite of the fact that it is not attacked by digestive enzymes!

Swartz says: "It is manifestly possible for fecal and soil bacteria to produce sugar from mannan (Cf. Sawamura (23); hence it is not unlikely that hemicelluloses of this group are inverted in the intestine through the activity of micro-organisms, and that the sugar so produced is absorbed and becomes a true source of energy for man, in spite of the resistance of mannan to the action of digestive enzymes. Further investigations to determine its exact nutritive value seem highly desirable!" In conclusion, "In considering the proper place in the dietary for marine algae, lichens, and similar substances, we must not disregard the possibility of their having a valuable function entirely aside from the question of energy production. As Oshima (20) points out, they may be valuable for their inorganic salts. The non-irritating, laxative properties

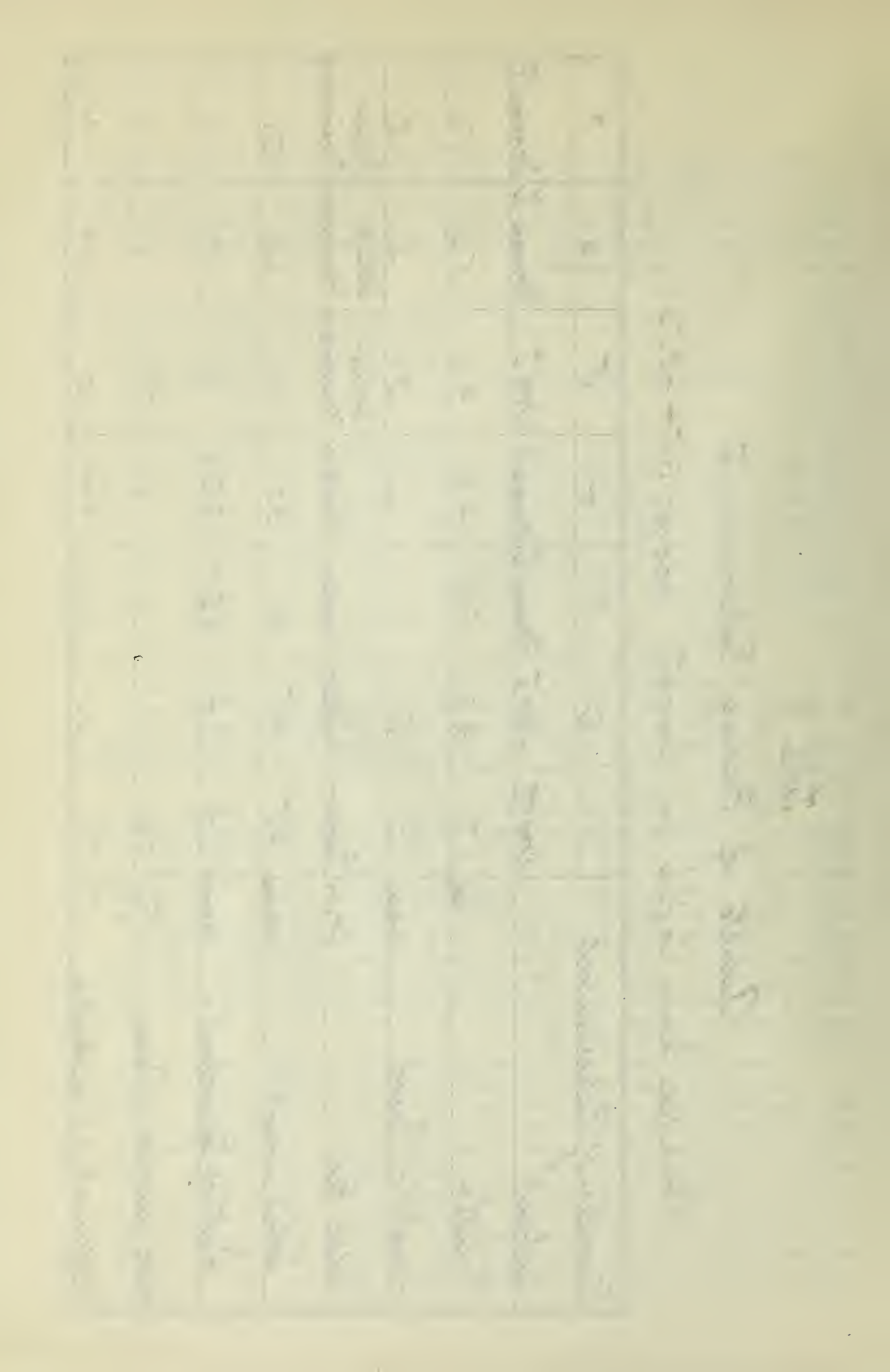
Fig. Sheet, page 321.

Table 1

Results of Feeding Experiments

Compiled from Table in Swartz' pages 354-356

Number of Experiments		1	2	3	4	5	6	7
Subject		Dog II	Dog I	Woman Z	Woman Z	Dog I	Woman Z	Woman P
Weight	kg	7	9.2	52	42	9.2	52	56
Length of Period	days	3	3	2	2	3	2	2
Salts Fed	form	Powder	Powder	Powder	Powder	Salep-Mannan	Salep Mannan	Salep-Mannan
Total weight -	grams	45	45	30	30	10	20	20
Dextrose Equivalent -	grams	34	34	22.7	22.7	9	18	18
Salts Recovered in Feces	%	30	0	0	6	90	0	0
Coefficient of Digestibility	%	70	100	100	94	10	100	100



of many species make them desirable adjuncts to the diet of persons with a tendency to constipation; and even if they disappear in a marked degree from the alimentary tract during the process of digestion, they may perhaps still play an important role as stimulants of intestinal activity, being in fact what Prausnitz (5) calls 'feces-forming foods! An illustration of this effect is afforded by the experiments in which salep powder was fed to dogs (Cf. pp. 352-3). The periods were equal in length and in one case the utilization of carbohydrates was equally good for all three; yet in the mid-period there is a decided increase in the bulk and weight of the feces (see table, p. 254, experiments 1 and 2), not more than one gram of which is by any possibility attributable to the cellulose of the salep powder, and in the other experiment, the increased amount of feces cannot be wholly accounted for by the amount of undigested carbohydrate present!

Simon (9) has recently found good utilization and glycogen formation in dogs from yeast gum which seems to be largely dextro-mannan.

EXPERIMENTAL PART

This experiment was undertaken for the purpose of supplementing the work done by Swartz on salep. It was planned to test the digestibility of salep by the human organism when it was taken in larger quantities than had hitherto been used, determining the actual amount passing through the body unused by an analysis of the feees. It was further proposed to compare its protein-sparing power with that of other carbohydrates by the determination of the urinary nitrogen and the nitrogen balance, when a portion of these carbohydrates was replaced quantitatively by salep.

The subjects were two healthy young women, [redacted], weighing 70 kilograms each, doing regular college work, including several hours a day in the laboratory. A previous feeding experiment had shown good utilization of carbohydrates by both persons.

It was decided to have a fore-period of 4 days, during which a basal ration was to be used, a mid-period of 4 days (subsequently reduced to 3) during which a portion of the carbohydrate of the basal ration was to be replaced by the salep under investigation, followed by an after-period like the fore-period.

A basal ration was selected , as nearly cellulose-free as possible. This was done in order that the utilization of the salep might not be interfered with by indigestible substances in the alimentary tract, and that the nitrogen-free extract in the feces might be reduced to a minimum. Atwater and Bryant (11) give the coefficient of digestibility of the carbohydrate of such a diet as 98 per cent. A portion of the carbohydrate was supplied by tapioca, which is almost pure starch. The grape juice was added for its mineral constituents and its palatability. Table 8 shows the basal ration as used, supplying approximately 2500 calories.

"Sheffield Farms" milk was used, obtained daily in quart bottles. Each morning the milk was thoroughly mixed and weighed out for each person for the day, a portion being taken from each day's supply to form a composite sample for analysis. This sample, preserved with formaldehyde, was kept in the refrigerator,the one in the laboratorybeing of the Bohn

Table 2

Dietary – Basal Ration

Food	I[1]									II[2]			
	Weight	Protein		Fat		Carbohydrate		100 calorie portion	Total calories	Nitrogen		Protein	
	grams	%	grams	%	grams	%	grams	grams		%	grams	%	grams
Milk	1305	3.3	42	4	52.2	5	65.25	145	900	0.51	6.656	3.188	41.6
Butter	39	1	0.39	85	34	—	—	13	300	0.16	0.06	1.[illegible]	0.39
Sugar	88	—	—	—	—	100	88.	25	352	—	—	—	—
Tapioca	75	0.4[4]	0.3	0.1[4]	0.75	88[4]	66	28.2	265.9	0.0797	0.059	0.498	0.37
Meat {Beef, loin, rump, edible portion}	96	21.3	20.44	7.9	7.[illegible]7	—	—	64	150	3.976	3.816	24.85	23.85
Crackers	107	9.8	10.48	9.1	9.74	73.1	78.22	24	442.45	1.942	2.078	12.13	12.99
Grape Juice	130	0.52[3]62	0.684	—	—	14.5[3]	18.85	166.3	78.14	0.0842	0.109	0.526[3]2	0.68
Total	1840		74.294		104.26		316.32		2488.5		12.77		79.88

[1] Analyses from Sherman, Chemistry of Food and Nutrition. Appendix Table I. [2] Analyses by the writer
[3] Analysis from Welch Grape Juice Co. [4] Analysis from Bulletin 28. Office of Experiment Stations, U.S. Dept. of Agriculture.

cooled by ammonia,
Siphon type, maintaining a temperature near or frequently below the freezing point..

The butter for each person each day was weighed out and wrapped separately at the beginning of the experiment.

The sugar was weighed out as used.

The tapioca was Durkee's "Sago Tapioca" It was weighed air dry, and cooked each day. A portion from each box was saved, forming a composite sample for analysis.

The meat was lean round-steak. All the fat and connective tissue possible having been removed, it was ground. Portions for each person each day were weighed out and wrapped separately, that for the latter part of the time being preserved by freezing, and a sample saved for analysis.

The crackers were National Biscuit Company's "Uneeda Biscuit" the amount for each day being weighed out each morning, and a portion from each box reserved for a composite sample for analysis.

Welch's Grape Juice was used, and the volume corresponding to the required weight having been determined, it was measured out each day in a graduated cylinder.

The meals were regular and uniform.

Breakfast, about 8:30, consisted of crackers and butter, milk, and sometimes sugar.

Lunch, about 1, consisted of crackers and butter, tapioca, sugar, milk, and grape juice.

Dinner, about 6:30, consisted of meat, cooked in a very little butter, and the rest of the crackers, butter, tapioca, sugar, milk, and grape juice.

During the mid-period, salep replaced a portion of the tapioca. The purified salep-mannan was prepared from the tubers. They were soaked 24 hours in water, washed thoroughly, and ground in a meat chopper. Water was added in large volume, the mass allowed to stand over night, and then the dissolved mannan filtered off through cheese cloth. As this was found to contain some starch, it was digested with filtered saliva (or malt diastase) until free from starch, and then poured into three times its volume of 95 per cent alcohol. A voluminous, flocculent, and somewhat fibrous precipitate formed, which was filtered off, washed in alcohol and then in ether, and dried in a vacuum dessicator. This darkens in drying, but if redissolved in hot water, precipitated in alcohol, then transferred to absolute alcohol and allowed to stand several days for thorough dehydration, filtered, washed with ether, and dried in a vacuum, a rather coarse white powder is obtained. This swells up readily in water, but dissolves very slowly to a semi-transparent mucilaginous solution. The salep-mannan may be prepared from the commercial salep powder in the same way.

Table 3 shows the amount of salep used and the dextrose equivalent.

Table 4 shows the slight changes in protein and carbohydrate during the salep period.

It was intended to replace as much as possible of the tapioca by salep, and the amounts of salep were as great as could be ingested by these subjects without such distaste as to imperil the continuance of the experiment. ¶ The first day of the mid-period the commercial salep powder was used, cooked with milk into a kind of pudding, but it was not satisfactory. It had a rather unpleasant odor and taste, and was slightly nauseating. It was very gummy in the mouth and somewhat gritty, giving a decidedly unpleasant sensation. The second day, the thoroughly purified

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Table 3

Salep

Day	Material Used	Weight grams	Dextrose Equivalent %	Dextrose Equivalent grams	Nitrogen %	Protein grams
First	Commercial Powder	25	76.9	19.23	0.6309	0.9856
Second	Purified - Starch-free	40	84.89	33.96	0.62	1.55
Third	Partly Purified	10	79.66	7.966	0.6224	0.389
Totals				61.156		
Average				20.384		

Table 4

Salep Period

Food	First Day			Second Day			Third Day		
	Weight grams	Protein grams	Carbohydrate grams	Weight grams	Protein grams	Carbohydrate grams	Weight grams	Protein grams	Carbohydrate grams
Milk	1305.	41.6	65.25	1305.	41.6	65.25	1305.	41.6	65.25
Butter	39.	0.39	—	39	0.39	—	39	0.39	—
Sugar	88.	—	88.	88.	—	88	88.	—	88.
Tapioca	50	0.249	44	35.	0.174	30.8	65	0.324	57.2
Meat	96	23.85	—	96	23.85	—	96	23.85	—
Crackers	107.	12.99	78.22	107	12.99	78.22	107	12.99	78.22
Grape Juice	130	0.684	18.85	130.	0.684	18.85	130	0.684	18.85
Salep	25	0.9856	19.23	40	1.55	33.96	10	0.389	7.966
Total		80.7486	313.55		81.238	315.08		80.227	315.486

substance (starch-free) was used. This was cooked in water, and though still exceedingly sticky, proved much more palatable than that of the previous day. The third day, a purified material, though not entirely starch-free, was used. It was given in the form of a thick gruel, flavored with grape juice, and was much easier to take in that way. However, owing to its very great thickening power and consequent volume, it was not possible to ingest so large an amount.

The urine was collected for each day separately, beginning at 8 a.m., and was preserved under toluol.

The feces were collected by periods, preserved under alcohol, and dried over a water bath as soon as possible. Lamp black was used for marking off the feces at the beginning of the experiment, but because of the dark colored feces produced by the grape juice, the other periods were marked by carmine.

Both subjects remained well on the diet. During the first days there was slight discomfort due probably to the difference between this and the previous diet, this one being less bulky, and containing practically no indigestible residue.

In spite of the small bulk, no difficulty was experienced with constipation. There was but one day with one subject (the third day of the fore period) when defecation did not take place. The increased bulk of the feces during the salep period compared with that of the fore-period was noticeable, as well as a slightly gelatinous consistency.

ANALYTICAL METHODS

Determinations of carbohydrate were made on the salep and feces.

The same form of salep that had been used in the diet was used for analysis.

The dried samples of feces for each period were ground to a fine powder and thoroughly mixed and samples taken from this for analysis.

For the determination of carbohydrate on the feces, samples of about 5 grams (accurately weighed) were hydrolyzed with 100 cc. of 2 per cent hydrochloric acid, boiling for 2 hours with a reflux condenser. Before boiling, about 4 grams of purified animal charcoal was added to each flask, preventing gumminess, and aiding materially in the subsequent filtering. After cooling, the material was neutralized with 20 per cent potassium hydroxide, and made up to 250 cc. This was filtered, and the filtrate, to which about 0.1 gram of salicylic acid was added, was kept in the refrigerator until used a day or two later.

The carbohydrate was determined as dextrose, using Allihn's gravimetric method (Sherman, Organic Analysis, pp. 100-103). The cuprous oxide was filtered through a Gooch crucible, ignited, and weighed as cupric oxide.

The carbohydrate determinations ~~were~~ on salep were made in the same way, the original samples varying from 0.9 to 2 grams.

Determinations of nitrogen were made on food, urine, and feces. Separate determinations were made on each of the foods except butter, the nitrogen of which, ~~had~~ in a previous experiment in this laboratory, had been found to agree closely with the value here used.

The Kjeldahl method (Hawk, Practical Physiological Chemistry, third edition, pp. 3 to 3) was used, a small piece of copper sulphate being added to facilitate the oxidation by the sulphuric acid, and

about 5 grams of potassium sulphate to raise the boiling point. In distilling, a piece of paraffin the size of a small pea, and a piece of zinc were added to prevent frothing and bumping. Care was taken to have the substances cold at the time of adding the saturated sodium hydroxide.

Samples were weighed out for analysis from the thoroughly mixed combined daily samples of milk, tapioca, and crackers. The ground meat was dried on a water bath, ground, and sampled. In the case of urine and grape juice, the determinations were made on samples of 5 cc. accurately measured with a pipette.

All determinations were made in duplicate, and many in triplicate.

DISCUSSION AND SUMMARY

Table 5 shows the amount of the feces by periods and the results of the analyses of these. The question of bulk is discussed later under Table 9.

The increased excretion of carbohydrate during the salep period, estimated as dextrose, is seen to total 1.65 grams for subject M, and 2.8 grams for subject H, giving a digestibility for salep of 97.29 per cent and 95.43 per cent for subjects M and H respectively. The per cent of carbohydrate excreted drops in the after-period to a point slightly higher than that of the fore-period with subject M, and slightly lower than that of the fore-period with subject H, who showed the less complete utilization of the salep.

The fecal nitrogen increases 0.35758 grams per day during the salep period for subject M, and 0.84255 grams per day for subject H. The per cent of nitrogen excretion by way of the intestine with subject M drops in the after-period to a point slightly below that of the fore-period, and with subject H to a point slightly above that of the fore-period. The digestibility of the nitrogen of the diet is seen to be about 95 per cent for both subjects on the basal ration, and 92.26 per cent for subject M, and 88.92 per cent for subject H during the salep period, which shows good utilization.

Table 6 shows that the digestibility of the carbohydrate of the basal ration was high, 99.84 per cent for both subjects, and that the digestibility of the salep was approximately 2.5 per cent less than this with subject M, and 4.4 per cent less with subject H. The digestibility of the salep thus averages about 96.4 per cent, or 3.5 per cent less than the digestibility of the carbohydrate of the basal ration.

Table 7 gives the volume and the nitrogen content of the urine by days and the average daily excretion of urinary nitrogen by periods. In subject M, the decreased nitrogen output during the salep period, compared with that of the two days just preceding, is noticeable, though the averages of the periods are exceedingly close. Both subjects show a decreased output of urinary nitrogen during the salep period. On the second day of the after-period there was a marked increase in the nitrogen output in both subjects, accompanied, in subject M by a conspicuous decrease in volume, and in subject H by a slight decrease in volume. What the disturbing influence was, we are unable to say, as we can find no factor nor factors capable of causing such a difference on this day. The figures in parenthesis beneath the averages for this period were obtained by taking the average of the other days of the period, and though

DISCUSSION AND RESULTS

Table 5
Feces

		M			H		
		Fore-Period 4 da.	Mid-Period 3 da.	After-Period 4 da.	Fore-Period 4 da.	Mid-Period 3 da.	After-Period 4 da.
Moist - Total Weight	gr.	257.	314.	241.5	149.3	369	182
Average wt. per day	gr.	64.25	104.66	60.375	37.325	123	45.5
Air Dry - Total Weight	gr.	57.	64.	60.	52.3.	81.5	54.
Average wt. per day	gr.	14.25	21.3	15.	13.	27.1	13.5
Moisture	%	77.82	79.62	75.16	64.97	77.93	70.33
Carbohydrate, Per cent	%	3.692	5.131	3.736	3.847	5.231	3.453
Total Weight	gr.	2.1044	3.2838	2.2416	2.012	4.264	1.865
Average wt. per day	gr.	0.52761	1.09461	0.56041	0.50225	1.4213	0.46625
Excess for Period (3 da)	gr.		1.65408			2.81879	
Salep Eaten (as dextrose)	gr.		61.156			61.156	
Digestibility of Salep	%		97.29			95.43	
Nitrogen, Per cent	%	4.481	4.671	4.35	4.503	5.269	4.7
Total Weight	gr.	2.555	2.989	2.61	2.355	4.294	2.538
Average wt. per day	gr.	0.63875	0.99633	0.65225	0.58875	1.4313	0.6345
Digestibility of Nitrogen	%	94.99	92.26	94.82	95.37	88.92	95.04

Table 6

Carbohydrate

		IV	V
Fore and after periods – carbohydrate eaten per day	grams	316.32	316.32
Average excretion carbohydrate per day	grams	0.54325	0.48437
Digestibility of carbohydrate	%	99.84	99.84
Mid-period – carbohydrate eaten per day	grams	314.705	314.705
Average excretion carbohydrate per day	grams	1.097	1.4213
Digestibility of carbohydrate	%	99.63%	99.54%
Average excess excretion over fore and after period, per day	grams	0.55736	0.93693
Total " " " " " " (due to skip)	grams	1.65408	2.81079
Carbohydrate as dextrose in salep eaten	grams	61.156	61.156
Digestibility of Salep	%	97.29%	95.43%



Table 7

Urine — Volume and Nitrogen Content

	Date, 1912	M			H		
		Volume cc	Total N grams	Average N grams	Volume cc	Total N grams	Average N grams
1	February 23	1560	9.612		2220	9.1	
2	February 24	1568	9.745	11.02	1690	10.09	10.212
3	February 25	1365	12.52		1622	12.13	
4	February 26	1655	12.18		1340	9.528	
5	February 27	1210	10.33		1000	9.099	
6	February 28	1110	11.625	10.956	1580	8.628	9.063
7	February 29	1950	10.925		2560	9.462	
8	March 1	1400	12.98		1725	11.74	
9	March 2	960	15.38	12.8925	1570	14.07	11.905
10	March 3	1250	10.85	(11.766)	1950	11.27	(11.8)
11	March 4	1280	11.47		1410	10.54	

still somewhat higher, are seen to be nearer the average output for the fore-period. The output of urinary nitrogen of subject H is seen to be lower throughout than that of subject M, but variations are in general in the same direction for both subjects. The unusually large volume of urine on the last day of the salep period is probably due to the increased intake of water on that day incident to the use of the salep in semi-liquid form.

Table 8 gives the nitrogen balance. This is seen to be positive throughout, except in the after-period of subject M, during which the abnormal day, above noted, occurred. If the average output of nitrogen in the urine for the other 3 days of the period be used for comparison, the balance for this period will be seen to be positive, as indicated by the figures in parenthesis. This table also shows that the increased output of nitrogen in the feces of the salep period was accompanied by a decreased output of metabolized nitrogen in the urine.

Table 9 shows the effect of the salep diet on the bulk of the feces, and on the moisture content. In the moist feces, the increase in average weight per day for the mid-period, compared with the average weight per day for the fore- and after-periods, is seen to be 67.98 per cent for subject M, and 197.1 per cent for subject H. With the air dry sample the increase is slightly less, 45.63 per cent for subject M, and 104.9 per cent for subject H.

The moisture content of the feces increased during the salep period, but not to a great extent, 4.09 per cent for subject M, and 15.2 per cent for subject H.

Thus this experiment seems to show that the human organism is able to utilize the mannan of salep, its average digestibility being 96.4 per cent, a coefficient only slightly less than the 98 per cent given by Atwater and Bryant (11) as the average digestibility of carbohydrates in a cellulose-free diet. Slight hydrolysis of the mannan may have been caused by the hydrochloric acid of the gastric juice, but it is known that cane sugar, much more easily hydrolyzed by acid than mannan, is not changed by the gastric juice to any great extent. In view of the experiments by Swartz (12) showing that salep-mannan is not attacked by digestive enzymes, but may be hydrolyzed by some of the bacteria of the alimentary tract, the most plausible explanation of this apparent high utilization of the salep seems to be that sugar was thus formed and was available to the body, but this point needs further elucidation.

This indirect evidence as to the utilization of salep, furnished by its almost complete disappearance from the alimentary tract, is confirmed by the nitrogen balance, which, with the single exception above noted, was positive. The fuel value of the diet was calculated as nearly as possible to the bodily requirement, and the reduction of the number of calories by the amount represented by the tapioca withdrawn would be expected to result in an increase in the output of urinary nitrogen, had this reduction not been compensated for by the salep ingested. The urinary nitrogen excreted during the salep period, instead of increasing, is appreciably less with both subjects during this period than on the days just preceding. Though this reduction in urinary nitrogen is accompanied by an increase in the fecal nitrogen during the salep period, it would seem to be the metabolized nitrogen excreted in the urine that is to be considered the criterion of availability. Thus McCollum (1), in discussing "Protein Metabolism: Growt and Repair" says: "Knowing the amount of nitrogen absorbed, our judgment concerning its value for replacing the endogenous loss should be based entirely on changes observed in the urine, viz., on the increase, if any, in the total nitrogen, and the change in the ratio between the nitrogen as

Table 8

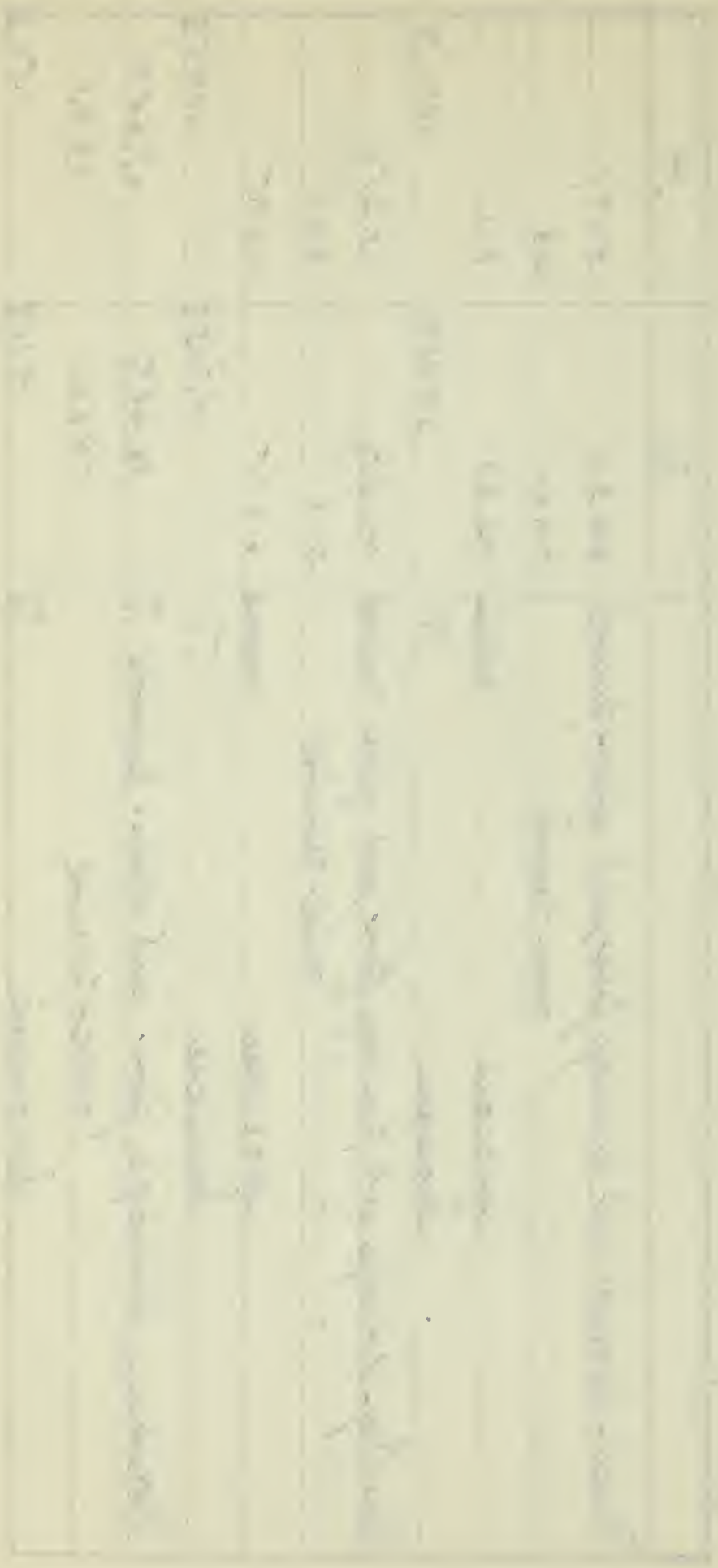
Nitrogen Balance

Period	N					I				
	Daily Output of N			Intake	Balance	Daily Output of N			Intake	Balance
	Urine grams	Feces grams	Total grams	grams	grams	Urine grams	Feces grams	Total grams	grams	grams
Fore	11.02	0.63875	11.659	12.77	+1.111	10.212	0.58875	10.801	12.77	+1.969
Fred	10.956	0.99633	11.952	12.92	+0.968	9.063	1.4313	10.494	12.92	+2.426
Adder	(11.766) 12.8925	0.6525	(12.4185) 13.5445	12.77	(+0.3515) −0.775	11.905	0.6345	12.54	12.77	+0.23

Table 9

Effect of Lalop Diet on Bulk of Feces and on Moisture Content

		In	H
Moist - Average wt. per day, fore- and after- periods		62.3 g.	41.4 g.
" " " " mid-period		104.66	123.
Increase	grams	42.23	81.6
Increase	%	67.98%	197.1%
Air Dry - Average wt. per day, fore- and after- periods		14.625 g.	13.25 g.
" " " " mid-period		21.3	27.1
Increase	grams	6.675	13.85
Increase	%	45.63%	104.5%
Moisture, Average, fore- and after- periods	%	76.49%	67.65%
" mid-period		79.62	77.93
Increase	%	+.09%	-5.2%



creatinine and the total nitrogen. Judged by this standard, the reduction in urinary nitrogen during the salep period apparently indicates that salep was more effective in preventing the use of body protein than was tapioca.

As stated above, the salep diet caused a much greater increase in the bulk of the feces than can be accounted for by the excreted carbohydrate alone, and, accompanying this, an increase in the fecal nitrogen. Mendel and Fine (2), following their studies on the utilization of vegetable proteins (wheat, barley, corn, legumes, and cotton seed) and of the proteins of extractive-free meat powder, discuss the influence of indigestible material on the fecal nitrogen. From the results of the work of Rieder (6), Tsuboi (7), Muller (4), Fritz Voit (8), and Rosenthal (3), Mendel and Fine consider that the feces resulting from a thoroughly digestible diet, such as meat, are almost solely of metabolic origin. From their own experiments they find that the addition of non-irritating, non-nitrogenous, indigestible material increases to a considerable extent the total output of fecal nitrogen, though the nitrogen intake does not vary. From these results, it was to be expected that if the salep were not digested, it would increase the output of fecal nitrogen, and if it were as well digested as the tapioca, it would not affect the fecal nitrogen. However, although the salep was almost as well digested as the tapioca, there is still a marked increase in the amount of nitrogen excreted in the feces. This increase in the fecal nitrogen, together with the reduced output of nitrogen in the urine, probably means that while salep is well utilized by the body, it is also a stimulant to intestinal activity, a "feces-forming food" as Prausnitz calls such substances, and that the increased nitrogen in the feces represents the residues of more abundant intestinal secretions.

CONCLUSIONS

1. This experiment confirms the earlier experiments as to the digestibility of salep. Considering the much larger amount ingested, the average digestibility of 96.4 per cent here obtained, almost as high as the digestibility of practically pure starch, compares favorably with the 100 per cent obtained by Swartz on smaller amounts.

2. The substitution, during three days, of an amount of salep equivalent to 20.4 grams of dextrose per day, for tapioca equivalent to 22.5 grams of dextrose per day, caused no increase in urinary nitrogen due to lowered fuel value of the diet, but, on the contrary, caused an appreciable decrease in the urinary nitrogen.

3. The ingestion of salep causes a noticeable increase in the bulk of the feces, which, in certain pathological conditions as constipation, might be of great advantage.

Other interesting lines of investigation suggested by these results are: the availability of salep as a food in cases of diabetes, the role of intestinal bacteria in the utilization of food, and the effect of salep on the creatine putput in the urine.

Such an investigation as this is of the greatest value to the person preparing to teach college work in nutrition and dietetics. The subject matter in this field is being continually affected by investigations of this type, and experience is needed to give criteria by which such contributions are to be judged. An adequate appreciation of the difficulties of such work, and of the many possibilities of error that must be guarded against if results of value are to be obtained, can only be acquired by first-hand experience.

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